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Evaluating the dose-dependent mechanism of action of trazodone by estimation of occupancies for different brain neurotransmitter targets

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Abstract

Trazodone is a drug that was introduced in the clinic almost 40 years ago. It is licensed to treat depression, but it is also commonly used off-label to treat insomnia. A recent study shows that it could be promising in preventing neurodegeneration in mice and clinical trials to assess its possible beneficial effects in dementia and Alzheimer's disease are expected to start soon in humans. In this study we describe the dose-dependent pharmacology of trazodone by carrying out a pharmacokinetic simulation aiming to predict the brain concentrations of trazodone for different drug dosing regimens and calculating occupancy for 28 different targets for which published trazodone binding data are available. Our study indicated that low doses of trazodone (typically 50 mg daily) should suffice to block specific receptors responsible for the hypnotic effect and for protective effect against neuroinflammation and neurodegeneration that could be beneficial in dementia. Higher doses are required for an antidepressant effect. The occupancy of specific receptors at therapeutic doses also explains peculiar side effects reported by trazodone (e.g. dry-mouth, priapism and hypotension).

Keywords:

Introduction

Trazodone was introduced in the early 1970s (Khouzam, 2017). Currently it is only licensed for treating depression, but it is commonly used off-label for other conditions, mainly to treat insomnia (Wong et al., 2017). Stahl reported that even if “trazodone was never officially approved as a hypnotic, nor marketed as a hypnotic, it nevertheless accounts for up to half of all prescriptions for hypnotics” (Stahl, 2013). Trazodone is also used off-licence to treat other conditions such as the behavioural and psychological symptoms of dementia in Alzheimer’s disease (AD) (Lopez-Pousa et al., 2008) and in fronto-temporal dementia (Lebert et al., 2004). In animals, it has previously shown benefit in models of Huntington’s disease (Kumar et al., 2011) by protecting neuronal-like cells from inflammatory insult (Daniele et al., 2015) and to prevent neurodegeneration (in mice) (Halliday et al., 2017). The latter study in particular provided promising results and clinical trials are expected to start soon to assess trazodone’s protective effect in dementia and AD.

Given that trazodone is only licensed for depression, there is need to know the dose required for treating the variety of other conditions for which it is used and the pharmacological mechanism of its action. The pharmacological activity of trazodone is dependent on the dose given and Stahl has tried to rationalise the dose-dependent

pharmacological effects of trazodone by discussing occupancy to different targets calculated using predicted plasma concentration of trazodone administered orally in different drug dosing regimens (Stahl, 2009).

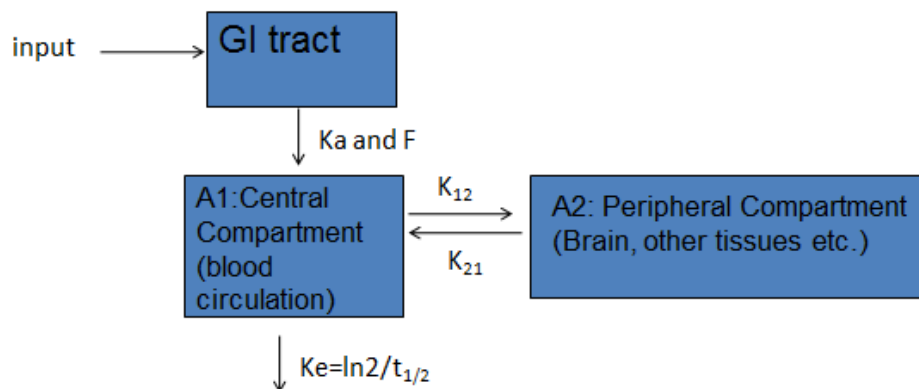
In the present study we wanted to improve the understanding of the mechanism of action of trazodone described in Stahl's analysis by (i) calculating receptor occupancy using brain concentration rather than the plasma level of trazodone; and (ii) considering a larger number of pharmacological targets. We also aimed to validate the use of a software called Berkley Madonna (BM) (Krause and Lowe, 2014; Berkeley Madonna, 2016). Thus we wanted to try to reproduce the experimental pharmacokinetic profile (PK) of trazodone and compare the performance of the model produced by BM with the methodology used by Stahl and co-workers (Lemaire et al., 2009; Stahl, 2009). Finally, we aimed to suggest a pharmacodynamic mechanism by which trazodone is able to reduce the levels of activating transcription factor 4 (ATF4) (a key mechanism in slowing neurodegeneration) without affecting the level of phosphorylated eukaryotic initiation factor 2 α (eIF2 α -P) as reported by Halliday and co-workers (Halliday et al., 2017).

With the prediction of occupancies we also suggest a likely dose of trazodone that could be used in future clinical trials to evaluate its possible protective effect in dementia and AD.

Method

A two-compartment pharmacokinetic (PK) model was developed using Berkeley Madonna (BM) beta version 9.0.123 (Berkeley Madonna, 2016; Krause and Lowe, 2014) in order to predict the pharmacokinetic profile of trazodone administered orally. A simple representation of the model developed in BM is shown in Figure 1 (the description of the compartments is similar to the structure of the model published by Lemaire and co-workers (Lemaire et al., 2009)).

Figure 1. The 2-compartment pharmacokinetic (PK) model developed for trazodone using BM.



Two differential equations were used for the modeling in BM: the first describes the absorption from the gastro-intestinal (GI) tract:

$$d/dt(\text{GI-tract}) = \text{input} - K_a * (\text{GI-tract})$$

where “input” accounts for the amount of trazodone taken orally in a specific time interval (24 hours in this study) and K_a is the constant of absorption from the GI tract to the central compartment. The amount of drug considered in the input function is the dose of trazodone multiplied by its bioavailability ($F=0.65$) (Truven Health Analytics, 2013) in order to account for the fraction of the administered dose that reaches the systemic circulation as unchanged drug. The “input” administration by the GI tract is modeled by the pulse function in BM (Krause and Lowe, 2014).

The second equation describes the distribution in the central compartment A1 and in the peripheral compartment:

$$d/dt(A1) = + K_a * (\text{GI-tract}) - K_e * A1 - K_{12} * A1 + K_{21} * A2$$

where K_e is the constant of elimination from the central compartment A1 (this is also equal to $\ln 2$ divided by the half life of elimination of trazodone – 7.3 hours in humans (Obach et al., 2008) thus a K_e of 0.095 h^{-1} assuming linear PK and first order elimination). K_{12} and K_{21} are the constants for the first order distributions of trazodone between the central and peripheral compartment. All the parameters of the model, the central (A1) and peripheral compartment (A2) are shown in Figure 1.

In order to solve the differential equation, the integration method Runge–Kutta 4 in BM was used; the time intervals to be used in the numerical solving of the differential equation system was 0.02 hours.

To create the trazodone PK model, the curve fit and sliders functions in BM were used for the estimation of K_a , K_{12} and K_{21} by modelling the experimental plasma concentration curve of a specific dose of trazodone published in the literature.

WebPlotDigitizer (Ankit Rohatgi, 2016) was used to extract the experimental data points of the trazodone plasma concentrations versus time measured after an oral administration of 50 mg of trazodone given to 6 individuals (having a mean weight of 70 Kg) from the plot in Figure 2 published by Gammans and co-workers (Gammans et

al., 1984). These points were imported as an external dataset in BM and this was used as a template to fit the PK model using the “curve fit” tool in BM by exploring different combination of K_a , K_{12} and k_{21} (K_e was instead set as 0.095 h^{-1} as explained in the methods section). BM allows also adjusting each parameter individually to better fit the curve using the sliders function.

The central compartment was approximated to represent the blood circulation compartment where trazodone is distributed as in the report published by Lemaire and co-workers (Lemaire et al., 2009) . In order to estimate the plasma concentration, the amount of trazodone in the central compartment was divided by the volume of distribution of trazodone in humans (36.4 L for a 70 Kg individual (Obach et al., 2008)). The human brain concentration was estimated considering the plasma/brain ratio measured experimentally in mice (the experimental $C_{u,\text{plasma}}/C_{u,\text{brain}}$ ratio reported in mice is 1.8 (Maurer et al., 2005) in this study we assumed that the plasma/brain ratio in humans is the same as the ratio measured in mice)

Different doses of trazodone were simulated (50 , 100 , 150 daily and 100 mg tds) and the integration interval was set as 24 hours.

The E max sigmoid model (Rosenbaum, 2011) was used to calculate the receptor occupancy. The formula is

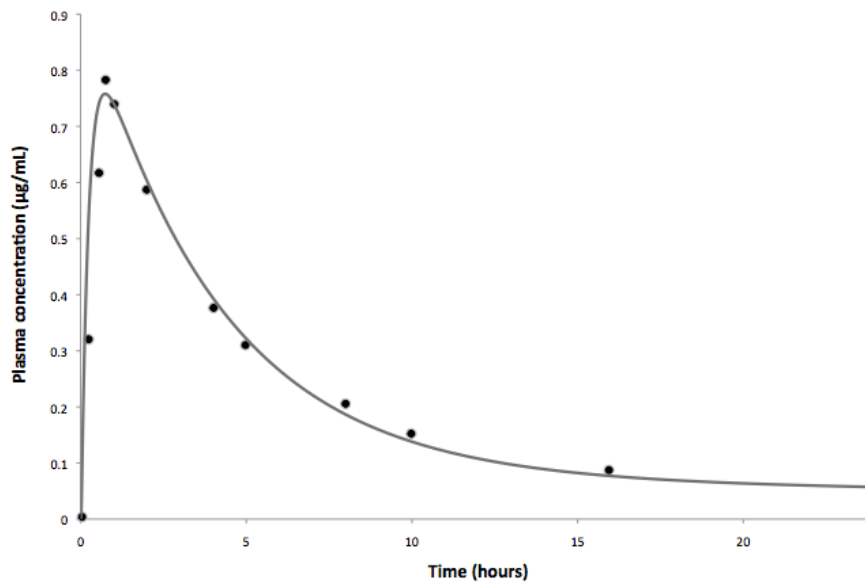
$$[\text{trazodone}]_{\text{brain}} / (K_i + [\text{trazodone}]_{\text{brain}})$$

where $[\text{trazodone}]_{\text{brain}}$ is the brain concentration of trazodone

Results

The experimental plasma concentration curve for trazodone was best modelled with a two-compartment pharmacokinetic (PK) model: the parameters for this PK model were the following : $K_{12}=0.134\text{ h}^{-1}$; $K_{21}=0.0313\text{ h}^{-1}$; $K_a = 4.319\text{ h}^{-1}$; $K_e = 0.095\text{ h}^{-1}$. The curve fit and sliders tools in BM were used to produce the PK model by fitting the data published by Gammans and co-workers (Gammans et al., 1984). The result is shown in Figure 2.

Figure 2. PK model fitting in BM. The data points were extracted from Figure 2 published by Gammans and co-workers (Gammans et al., 1984). The continuous line represents the PK fitted curve obtained using BM.



This modelling methodology seems suitable in the case of trazodone as can be seen with the good fit between the simulated curve produced with our model in BM and the experimental plasma values measured by Gammans and co-workers (Gammans et al., 1984). This is also in agreement with other studies showing that trazodone follows a two-compartment pharmacokinetic model (Lee and Desai, 2007; Lemaire et al., 2009).

The fact that our model shows that the K_{12} is 4.3 fold higher than K_{21} suggests that trazodone distributes substantially to the peripheral compartment (Figure 2). This was expected since trazodone is a basic lipophilic compound, thus this compound is predicted to bind to fat tissues and membranes (Schmitt, 2008).

The plasma concentration and the brain concentration curves for trazodone 50, 100, 150 daily and 100 mg tds predicted with BM are shown in Figures 3 and 4 respectively

Figure 3. Plasma concentration curves for trazodone 50, 100, 150 daily and 100 mg tds predicted with BM using the 2-compartment model created as described in the methods section.

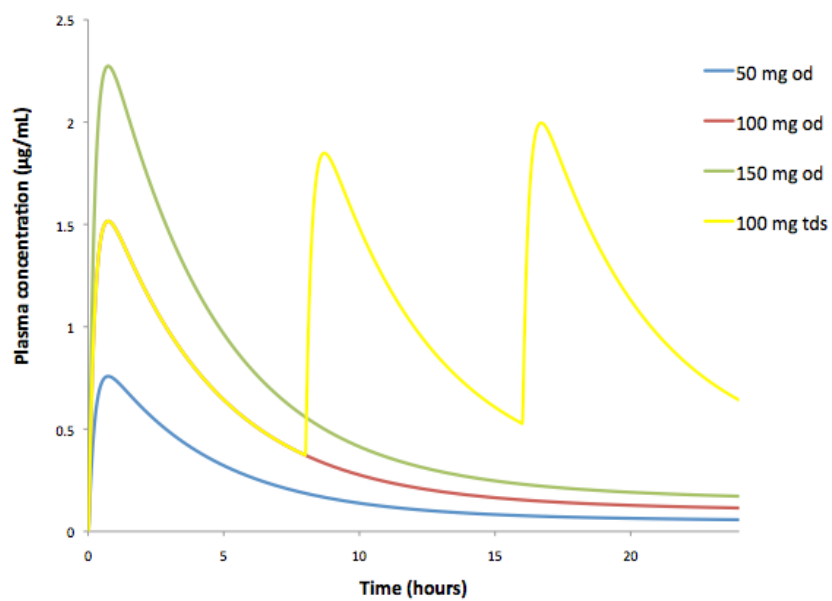
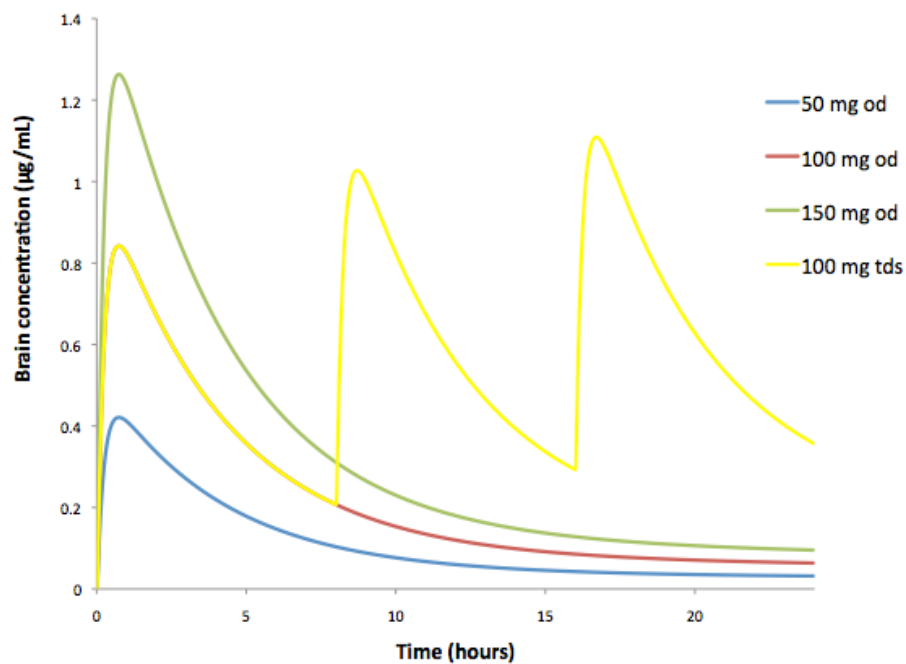


Figure 4. Brain concentration curves for trazodone 50, 100, 150 daily and 100 mg tds predicted with BM using the 2-compartment model created as described in the methods section.



The therapeutic (antidepressant) window of trazodone is said to be a plasma level of between 0.5 and 1.6 µg/mL whereas toxic effects are expected to be seen when the concentration of trazodone is above 4 µg/mL (Schulz and Schmoldt, 2003). The drug dosage regimen that best fits this window of plasma concentrations is seen when trazodone is given as 100 mg od or tds (Figure 2). When 50 mg od is given, the plasma level is for the most part below the therapeutic threshold. When 150 mg is given the

plasma concentrations are within the therapeutic window but approach the upper limit of the therapeutic window (Figure 2).

Given that the free plasma/brain ratio is expected to be 1.8 (assuming that the brain permeability of trazodone in mice (Maurer et al., 2005) is similar to the brain permeability in humans) the brain concentration is expected to be almost half than the plasma concentration (as can be seen by comparing Figure 2 and Figure 3).

We calculated the occupancy with the brain concentration using the E_{max} model described in the methods section; in order to calculate the occupancy we needed (i) the concentrations of trazodone in the brain for each drug dosage regimen and (ii) the binding affinity for the receptor or transporter (published in the literature). These values are reported in Table 1 and Table 2.

Table 1 shows the C_{max} concentrations in the plasma and brain for the three different drug dosage regimens taken from Figure 3 and 4. The brain C_{max} concentrations were used to calculate the occupancies since the pharmacological actions of trazodone originates by binding to the different targets (receptors and transporters) in the brain.

Table 1. C_{max} trazodone (in µg/mL or mg/L) concentrations predicted in the plasma and in the brain after the BM PK simulation of trazodone given as 50 mg od, 100mg od and 150 mg od. a:value taken from the curves in Figure 2; b: value taken from the curves in figure 3.

Dose	Plasma C _{max} ^a	Brain C _{max} ^b
50 mg daily	0.76	0.42
100 mg daily	1.5	0.83
150 mg daily	2.27	1.26

Table 2 reports the binding affinity of trazodone for key receptors and transporters obtained from the literature (Roth and Driscoll, 2011).

Table 2. Binding affinities (where data available) of trazodone for different transporters and receptors (trazodone is an antagonist/blocker for all the targets in the table except for 5-HT_{1A} receptor where it behaves as a partial agonist/agonist). Values taken from reference (Roth and Driscoll, 2011).

Protein	Ki (nM)
SERT	367.3
NET	>10000
DAT	>7000
5-HT _{1A} (*)	118
5-HT _{1B}	>10000
5-HT _{1D}	106
5-HT _{1E}	>10000
5-HT _{2A}	35.8
5-HT _{2B}	78.4
5-HT _{2C}	223.9
5-HT ₃	>10000
5-HT _{5A}	>10000
5-HT ₆	>10000
5-HT ₇	1782
α_{1A}	153
α_{1B}	ND
α_{2A}	728
α_{2B}	ND
α_{2C}	155
β_1	>10000
β_2	>10000
D ₁	3730
D ₂	4142
D ₃	ND
D ₄	703
D ₅	>10000
H ₁	220
H ₂	3290
H ₄	>10000
mAChRs	>10000
nAChRs	>10000

SERT: serotonin transporter; NET: norepinephrine transporter; DAT: dopamine transporter; 5-HT: 5-hydroxytryptamine(serotonin) receptors (different subtypes); α : alpha adrenergic receptors (different subtypes); β : beta adrenergic receptors (different subtypes); D1-5: dopamine receptors; H: histamine

receptors (different subtypes); mAChR muscarinic receptors; nAChRs: nicotinic receptors; ND: not determined. (*):Trazodone behaves as agonist/partial agonist for 5-HT_{1A} (Odagaki et al., 2005)

We calculated the occupancy for all the proteins (receptors and transporters) in Table 2 using the brain concentrations for the three drug dosage regimens in Table 1 and reported the results in Table 3.

Table 3. Occupancy calculated with the E max sigmoid model based on the brain concentrations C_{max} reported in Table 2.

Protein	Occupancy for dose of 50 mg daily	Occupancy for dose of 100 mg daily	Occupancy for dose of 150 mg daily
SERT	0.75	0.86	0.90
NET	< 0.10	<0.18	<0.25
DAT	< 0.14	<0.24	<0.33
5-HT _{1A} (*)	0.91	0.95	0.97
5-HT _{1B}	< 0.10	<0.18	<0.25
5-HT _{1D}	0.91	0.95	0.97
5-HT _{1E}	< 0.10	<0.18	<0.25
5-HT _{2A}	0.97	0.98	0.99
5-HT _{2B}	0.94	0.97	0.98
5-HT _{2C}	0.83	0.91	0.94
5-HT ₃	< 0.10	<0.18	<0.25
5-HT _{5A}	< 0.10	<0.18	<0.25
5-HT ₆	< 0.10	<0.18	<0.25
5-HT ₇	0.39	0.56	0.66
α _{1A}	0.88	0.94	0.96
α _{1B}	ND	ND	ND
α _{2A}	0.61	0.75	0.82

α_{2B}	ND	ND	ND
α_{2C}	0.88	0.94	0.96
β_1	< 0.10	<0.18	<0.25
β_2	<0.10	<0.18	<0.25
D ₁	0.23	0.37	0.48
D ₂	0.21	0.35	0.45
D ₃	ND	ND	ND
D ₄	0.62	0.76	0.83
D ₅	< 0.10	<0.18	<0.25
H ₁	0.84	0.91	0.94
H ₂	0.26	0.40	0.51
H ₄	< 0.10	<0.18	<0.25
mAChRs	< 0.10	<0.18	<0.25
nAChRs	< 0.10	<0.18	<0.25

ND: not determined. (*):Trazodone behaves as agonist/partial agonist for 5-HT_{1A}

(Odagaki et al., 2005).

Discussion

BM is able to reproduce PK simulations published in the literature

The plasma concentration curves predicted with BM for the trazodone doses of 50 mg od, 100 mg od and 100 tds (in Figure 3) are very similar to the plasma concentration curves for the same dosage regimens published by Stahl (Stahl, 2009). This demonstrates the strength and reliability of the methodology used in our study and validates our PK model created with BM since this produced results very similar to published PK data (Lemaire et al., 2009; Stahl, 2009).

Trazodone has a half-life of 7.3 hours (Obach et al., 2008) and it requires frequent administrations in order to provide a sustained exposure: the ideal time interval of administration for a drug is an interval as close as possible to the half-life of the drug administered if the oral IR formulation is given (Rosenbaum, 2011). Thus for trazodone standard release, the ideal oral dosage regimen is three times daily. This can be seen in Figure 3 and 4. In order to provide a better exposure over time during the day, a drug dosage regimen such as 100 mg tds should be chosen rather than a once daily administration. This fact, in combination with the risk of toxic effect seen when the C_{max} is reached (especially when high doses are administered) explains why the

manufacturer suggests administrating high doses of trazodone in divided doses (Concordia International, 2016). An alternative to the administration of trazodone in divided doses during the day is the once daily administration of a MR formulation of trazodone (Fagiolini et al., 2012; Lemaire et al., 2009; Stahl, 2009).

Dosing trazodone for insomnia

Trazodone is used widely off-label in a low-dose as hypnotic for the treatment of sleep disorders (Stahl and Stahl, 2011). Trazodone's short half-life is advantageous to treat insomnia, because its daytime sedation is minimal when trazodone is given only at night and at low doses, in agreement with the rapid decrease of the plasma and brain concentration curves for the single dose administration (Figures 3 and 4).

Different studies were performed to determine the optimal dose of trazodone to treat insomnia.

The first study in the respect was carried out by Muratorio and co-workers showing that low doses of trazodone (50 mg daily) were not effective as hypnotic in 4 non-depressed patients whereas higher doses (> 250 mg daily) given to depressed patients helped the patients to sleep (Muratorio et al., 1974). In contrast, Karniol and co-

workers showed that in 10 healthy volunteers were drowsier when taking a dose of 0.33 mg/Kg (~ 25 mg daily considering an average weight of the patients of 70 Kg) rather than a dose of 0.57 mg/Kg (~ 50 mg daily) (Karniol et al., 1976). Both these previous studies should be interpreted carefully given the small number of patients involved. In contrast, another study involving 75 patients analysed the effect of dose on the treatment of insomnia associated with depression and concluded that a daily dosage of 50-100 mg (at night) improved sleep disorders, particularly when given at a dose of 100 mg (Mashiko et al., 1999). This finding was confirmed by another study in depressed insomniacs (Saletu-Zyhlarz et al., 2002). Recently Savarese and co-workers performed a retrospective cross-sectional study on 33 patients treated with trazodone given at different doses to treat insomnia for 3 months (Savarese et al., 2015) and reported that when patients were given a daily dose of 25-75 mg (at night) there were 37.93% , 31.03% and 20.68% of responders when the night dose of trazodone was 25 mg, 50 mg and 75 mg respectively. When the dose was 100 mg or 150 mg daily at night, responder rates were only 10.43% and 0% respectively (Savarese et al., 2015). Taken together these studies show that a dose between 25mg and 100 mg seems to have a significant hypnotic effect that justifies the use of trazodone for insomnia at low doses.

Only one randomized parallel-group, double-blind study has been carried out for trazodone in non-depressed insomniacs. This established the hypnotic efficacy of trazodone in comparison with zolpidem and placebo and concluded that trazodone given 50 mg daily for 2 weeks was an effective hypnotic for the short-term treatment of patients with primary insomnia, being only slightly less efficient than zolpidem (Walsh et al., 1998). Alongside these studies is the observation that the typical off-licence hypnotic dose of trazodone in non-depressed patients is 50 mg at night. We performed a PK simulation with such dose (50 mg daily) to better understand the pharmacological mechanism behind the hypnotic effect of trazodone. This dose afforded after approximately one hour following oral administration (Figure 3 and 4), a C_{max} in the brain of 0.42 mg/L (Table 1). With this concentration, the predicted occupancy for SERT is 75%, whereas the serotonin receptors 1A,1D,2A and 2B are expected to be more than 90% occupied. 5HT_{2C} receptors are expected to show an occupancy of 83% whereas 5HT_{1B} and 1E are expected to show an occupancy less than 10% (Table 3).

The calculated occupancy for SERT is too low to achieve an antidepressant activity when a dose of 50 mg is given, since this transporter should be almost fully inhibited in order to exert a pharmacological action (Stahl, 2009). The hypnotic/anxiolytic effect at

low doses of trazodone can be understood by taking into account the occupancy predicted in our analyses as an antagonist for (i) 5-HT_{2A} receptor (97%); (ii) alpha_{1A} receptor (88%) and (iii) H₁ receptor (84%). The antagonism of each these receptors being known to cause an hypnotic effect (Stahl, 2009; Fagiolini et al., 2012). In addition, the activation of the 5-HT_{1A} (91% occupancy as agonist/partial agonist) is likely to contribute to the anxiolytic effect of trazodone at these doses (Odagaki et al., 2005).

Less is known about the 5-HT_{1E} receptor, however it has been proposed that it could regulate memory given that it is highly localised in the cortex, hippocampus, and olfactory bulb (Bai et al., 2004). The fact that trazodone does not block this receptor suggests that it could be used as hypnotic without affecting memory. This might suggest that trazodone can be safely prescribed in patients affected with dementia not least because trazodone does not to any extent block muscarinic receptors (table 3). A recent randomised double-blind and placebo-controlled study in AD patients confirmed in fact that trazodone (given with a dose of 50 mg at 10:00 PM for 2 weeks) was effective as hypnotic and did not have any effect on cognition using different rating scales (Camargos et al., 2014).

Dosing trazodone for depression

Two clinical studies investigated the optimal dosing of trazodone for treating depression: Mukherjee and Davey compared the treatment of trazodone dosed as 25 mg tds against 50 mg tds and reported the superiority of the latter dose regimen in treating this condition (Mukherjee and Davey, 1986). Mihara and co-workers reported a significant linear relationship between the steady-state plasma concentration of trazodone and the percentage of patients improving depression as assessed by the Montgomery–Åsberg Depression Rating Scale (MADRS) (Mihara et al., 2002).

Trazodone probably acts as antidepressant by different pharmacological mechanisms such as antagonism of the serotonin transporter (SERT), alpha-2 adrenoreceptor, 5-HT_{2A} and 5-HT_{2C} receptors, as well as stimulation of the 5-HT_{1A} (Stahl, 2009; Fagiolini et al., 2012).

Drugs that block the re-uptake of serotonin increase the concentration of this neurotransmitter in the synaptic cleft. This is a well-known mechanism of action for the selective serotonin reuptake inhibitors (SSRI): excess of serotonin in the synapse is thought to act on the post-synaptic 5-HT_{1A} receptor providing antidepressant action (Stahl, 2009; Stahl, 2008). SSRIs do not block post-synaptic 5-HT receptors and are

associated with side effects such as insomnia, anxiety and sexual dysfunction (Stahl, 2008). Serotonin agonism on the 5HT_{2A} and 5-HT_{2C} post-synaptic receptors is the mechanism for this (Stahl, 2009; Stahl, 2008). The potential advantage of drugs such as trazodone is that, in addition to blocking SERT, trazodone is an antagonist at 5HT_{2A} and 5-HT_{2C} receptors. 5-HT_{2C} antagonism is observed with other drugs such as mirtazapine and agomelatine (Stahl, 2009; Stahl, 2008). Each of these non-SSRI drugs are known to exert antidepressant activity with a much reduced risk of anxiety or sexual dysfunction.

In addition to this, it has been postulated that simultaneous 5-HT_{2A} and 5HT_{2C} antagonism combined with SERT inhibition might also potentiate antidepressant effect and improve tolerability (Fagiolini et al., 2012; Stahl, 2009).

As can be seen in Table 3 the occupancy for SERT is predicted to be 86% when trazodone is given as 100 mg daily and to 90% when trazodone is given as 150 mg daily. As expected, at these doses trazodone blocks almost completely the 5HT_{2A} and 5-HT_{2C} receptors thus, in theory, reducing the risks of sexual dysfunction and anxiety related side-effects (Stahl, 2009). The fact that high doses of trazodone are more effective in treating depression than low doses (Mukherjee and Davey, 1986; Mihara et al., 2002), suggests that the block of SERT (that is only significantly achieved when high

doses are given as explained above) is more important than the block of alpha-2 adrenoceptor, 5-HT_{2A} and 5-HT_{2C} receptors or the stimulation of the 5-HT_{1A} receptor for treating depression. These receptors show a high occupancy at lower doses (Table 3).

Different studies report that trazodone usually decreases REM sleep (Aton et al., 2009) (Brogden et al., 1981) (Mendelson, 2005) (Yamatsu et al., 1974; van Bommel et al., 1992). However, to our knowledge only one study investigated the effect of the dose of trazodone to the amount of rapid eye movement (REM) during sleep following administration of this drug (Yamatsu et al., 1974). According to this investigation the suppression of REM is proportional to the dose of trazodone administered (Yamatsu et al., 1974). We suggest that this could be correlated to the fact that at high doses of trazodone, the increased concentration of serotonin in the synaptic cleft (as a result of SERT-block) activates 5-HT_{1B} receptors. The agonist activation of this receptor has been shown to decrease REM during sleep (Boutrel et al., 1999): the fact that trazodone does not block this receptor (Table 3) could explain the fact that when serotonin concentration increases following SERT block by high doses of trazodone, the effect is a suppression of REM and this hypothesis would be confirmed by the trazodone's dose-dependent suppression of REM. These findings support the fact that low doses of

trazodone (typically 50 mg at night) should be preferred for treating insomnia, since the quality of sleep would be improved given that REM phases would not be majorly affected.

Dosing trazodone for possible neuroprotection in humans

Halliday and co-workers recently proposed a possible beneficial use of trazodone in dementia and AD by showing that this drug is able to reverse unfolded protein response (UPR)/integrated stress response (ISR) activation induced by UPR stressors such as tunicamycin. The authors showed in particular that trazodone was able to reduce the levels of activating transcription factor 4 (ATF4) without affecting the level of phosphorylated eukaryotic initiation factor 2 α (eIF2 α -P) (Halliday et al., 2017). However, the authors of this study did not explain the mechanism by which trazodone produced this effect. Given that it has been showed that p38 activates the expression of ATF4 in tunicamycin-induced endoplasmic reticulum stress-related PERK/eIF2 α /ATF4 pathway (Jiang et al., 2014) and also that 5-HT_{2A} agonism activates the mitogen-activated protein kinase p38 (Kurrasch-Orbaugh et al., 2003), it is evident that trazodone's antagonist activity on the 5-HT_{2A} receptor is expected to decrease the activity of p38 and this should decrease also the formation of ATF-4. Thus we suggest that 5-HT_{2A} blockade could have a protective role against dementia and AD and explain

the effects described in the recent study (Halliday et al., 2017). Our hypothesis has been also confirmed by the fact that there is partial reduction of neuro-protective effect in presence of the 5-HT_{2A} receptor agonist (R)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (R-DOI) (Daniele et al., 2015). The latter ligand displays a high affinity towards 5-HT_{2A} (K_i=3.36 nM) (Knight et al., 2004), approximately 10- fold stronger than the affinity of trazodone for the same receptor. In addition, the decreased activity of p38 as result of 5-HT_{2A} block is expected to decrease the expression of nuclear factor kappa B (NF-kappa B), that is an important transcription factor in inflammation (Olson et al., 2007). Moreover, trazodone is expected to have beneficial towards dementia and AD because, as a 5-HT_{1A} agonist it is expected to lead to ERK1/2 activation that is known to increase the protein expression of brain-derived neurotrophic factor (Polter and Li, 2010; Masson et al., 2012; Daniele et al., 2015). Also the block of H₁ and alpha-1 receptors are both expected to decrease the expression of NF-kappa B (Bakker et al., 2001; Gupta et al., 2009; Perez et al., 2009)

Given that 5-HT₁, 5-HT_{2A}, H₁ and alpha₁ receptors are saturated with a dose of 50 mg daily (Table 3), our study suggests that this dose might suffice for observing neuro-protective effects towards dementia/AD and we suggest that it will be reasonable to use this dose for future clinical studies of trazodone in dementia/AD.

Rationale for the presence of unanticipated side effects of trazodone

The fact that trazodone displays minimal central or peripheral occupancy for muscarinic receptors at these doses makes this drug suitable for treating patients with glaucoma, angina, prostatism, constipation, and dementia.

The central occupancy of the receptors calculated in our study can also be used to understand the side-effects reported by patients taking trazodone. In particular, we explain how the occupancy for the different receptors for trazodone can explain three peculiar side effects of trazodone (i) hypo-salivation; (ii) hypotension; (iii) priapism.

It is clear from Table 3 that trazodone does not block the muscarinic receptor, thus hyposalivation is not mediated by antagonism of acetylcholine. Lung and colleagues reported that salivation is also controlled by alpha receptors: in particular hypersalivation can be caused by alpha 1-agonists and/or alpha-2 antagonists, whereas hypo-salivation is caused by alpha 1 antagonists and/or alpha 2 agonists (Lung, 1994). This is also supported by one of the theories according to which the clozapine-induced hypersalivation is mediated by the block of the alpha-2 adrenergic receptors (Corrigan et al., 1995; Szabadi, 1996). The binding data reported in Table 2 show that trazodone has a stronger affinity for the alpha-1A receptor than the alpha-2 receptors (153 nM

and 441.5 nM (average value of the affinity for the α_2C and α_2A receptors) respectively). This is also reflected with the occupancy (Table 4). This binding profile could therefore explain why trazodone can cause hypo-salivation (dry mouth).

A relatively frequent side effect of trazodone is hypotension. The specific binding profile of trazodone in respect to alpha receptors can also help to explain this side effect. It is known that alpha-1 agonists and alpha-2 blockers cause hypertension whereas alpha-1 antagonists and alpha-2 agonists cause hypotension (Reid, 1986). Using a similar reasoning to provide the explanation for the hyposalivation, the fact that the block of alpha-1 receptor is stronger than alpha-2 receptor can also explain the hypotension sometimes seen with trazodone.

There have been several reports of priapism associated with the use of trazodone (Hayes and Kristoff, 1986). Our study agrees with published studies that proposed that the effect is related to blockade of alpha-receptors in the absence of sufficient antimuscarinic activity (Patel et al., 1996). This criteria is fulfilled by the pharmacological profile of trazodone. Notably yohimbine blocks the alpha-2 receptors and has been studied for potential treatment for erectile dysfunction (Andersson and Stief, 2001).

Conclusion

In this study we have validated the use of BM for PK simulations as we were able to reproduce PK simulations of trazodone published in the literature. We have shed some more light on the mechanism of action of trazodone in the treatment of different conditions (depression, insomnia and a possible use as protective treatment for dementia/AD). Our study indicates that low doses (typically of 50 mg daily) should be used for treating insomnia and such doses will have a minimal influence on REM sleep, and possibly to further investigate a pharmacological protection of trazodone for neurodegenerative conditions such as dementia/AD.

Our findings are corroborated by the prediction of brain concentrations of trazodone using different drug dosing regimens and the prediction of occupancy for 28 different targets for which published trazodone binding data are available. The occupancy of specific receptors is also able to explain the rationale for the presence of specific side effects reported for trazodone.

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